

PATENT  
09/849,022  
Docket 091/005

CLAIM AMENDMENTS

1. *(Currently Amended)* A method for producing a population of genetically altered human embryonic stem (hES) cells, comprising:
  - a) obtaining a culture comprising ~~human embryonic stem~~ hES cells proliferating ~~on an extracellular matrix instead of feeder cells in a culture environment essentially free of feeder cells but comprising an extracellular matrix;~~ and
  - b) ~~transfecting at least some of the cells in the composition with a polynucleotide;~~ transfecting the cells with a polynucleotide while being cultured in the culture environment, wherein the polynucleotide comprises a protein encoding region operably linked to a promoter that promotes transcription of the encoding region while the cells are undifferentiated,  
thereby producing genetically altered stem cells ~~that are hES cells that express the protein while~~ undifferentiated.
2. *(Original)* The method of claim 1, further comprising preferentially selecting cells that have been genetically altered with the polynucleotide.
3. *(Previously presented)* The method of claim 1, wherein the human embryonic stem cells are cultured in an environment comprising extracellular matrix components and a conditioned medium produced by collecting medium from a culture of feeder cells.
4. *CANCELLED*
5. *(CANCELLED)* The method of claim 1, wherein the polynucleotide comprises a protein encoding region operably linked to a promoter that promotes transcription of the encoding region in an undifferentiated embryonic stem cell.
6. *(Previously presented)* The method of claim 1, wherein the polynucleotide is selected from an adenoviral vector, a retroviral vector, and a DNA plasmid complexed with positively charged lipid.
7. *CANCELLED*

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8. *(Currently Amended)*

A cell population comprising undifferentiated human embryonic stem (hES) cells  
~~some of which have been genetically altered, wherein the population consists essentially of~~  
~~human cells~~  
expressing a protein from a heterologous polynucleotide in which an encoding region for the  
expressed protein is operably linked to a promoter that promotes transcription of the encoding  
region while the hES cells are undifferentiated.

9. *(Currently Amended)*

A cell population comprising undifferentiated human embryonic stem (hES) cells  
~~some of which have been stably transfected, wherein the population consists essentially of~~  
~~human cells~~  
stably transfected so as to express a protein from a heterologous polynucleotide in which an  
encoding region for the expressed protein is operably linked to a promoter that promotes  
transcription of the encoding region while the hES cells are undifferentiated.

10 to 12. CANCELLED

13. *(Currently amended)* The cell population of ~~claim 10~~ claim 8, in which at least 90% of the undifferentiated ~~pPS~~ hES cells have been genetically altered.

14. CANCELLED

15. *(Currently amended)* The cell population of claim 9, in which at least 90% of the undifferentiated ~~pPS~~ hES cells have been stably transfected.

16. *(Previously presented)* A method for producing genetically altered differentiated cells, comprising differentiating the cells of claim 9.

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17. *(Currently amended)* A method for producing genetically altered differentiated cells, comprising:
- a) obtaining a culture comprising human embryonic stem cells proliferating ~~on an~~ extracellular matrix instead of feeder cells in a culture environment essentially free of feeder cells but comprising an extracellular matrix; and
  - b) transfecting at least some of the cells in the composition with a polynucleotide, thereby producing genetically altered cells; and
  - c) causing the genetically altered cells to differentiate into a population of neural cells or hepatocytes.
18. *(Currently amended)* The method of ~~claim 8~~ claim 16, whereby the genetically altered cells are differentiated into neural cells.
19. *(Currently amended)* The method of ~~claim 8~~ claim 16, whereby the genetically altered cells are differentiated into hepatocytes.
20. *(Currently amended)* The method of claim 17, whereby the ~~genetically altered cells are differentiated into~~ differentiated cell population is over 50% neural cells.
21. *(Currently amended)* The method of claim 17, whereby the ~~genetically altered cells are differentiated into~~ differentiated cell population is over 50% hepatocytes.
22. *(Previously presented)* The method of claim 1, wherein the polynucleotide encodes a drug resistance gene.
23. *(Previously presented)* The method of claim 2, wherein the selecting comprises culturing the cells in the presence of a drug to which genetically altered cells in the population are resistant.

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24. (New) The method of claim 1, wherein said promoter is selected from the EF1a promoter and the PGK promoter.
25. (New) The cell population of claim 8, wherein said promoter is selected from the EF1a promoter and the PGK promoter.
26. (New) The cell population of claim 9, wherein said promoter is selected from the EF1a promoter and the PGK promoter.
27. (New) The cell population of claim 8, which consists of human cells.
28. (New) The cell population of claim 9, which consists of human cells.
29. (New) The cell population of claim 8, wherein the protein is a factor that supports growth of the hES cells.
30. (New) The cell population of claim 29, wherein the protein is a fibroblast growth factor.
31. (New) The cell population of claim 8, wherein the protein is a detectable label.
32. (New) The cell population of claim 31, wherein the label is a fluorescent label.
33. (New) The cell population of claim 32, wherein the label is selected from luciferase and green fluorescent protein (GFP).
34. (New) The cell population of claim 31, wherein the label is a cell surface protein detectable by antibody staining.
35. (New) The cell population of claim 31, wherein the label is an enzyme.
36. (New) The cell population of claim 35, wherein the label is selected from alkaline phosphatase,  $\beta$ -galactosidase, and neophosphotransferase.